

GLYCYRRHETINIC ACID-DERIVATIVES: ASSESSMENT OF TARGET SELECTIVITY



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INTRODUCTION

The activity of glucocorticoids is regulated on a cell-specific level by 11 β -hydroxysteroid dehydrogenase (11 β -HSD) enzymes. Two distinct enzymes are known: 11 β -HSD1 and 11 β -HSD2.

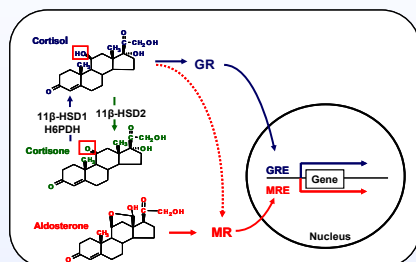


Fig.1
11 β -HSD1 converts inactive 11ketoglucocorticoids to the active 11 β -hydroxyglucocorticoids.
11 β -HSD2 catalyzes the reverse reaction.

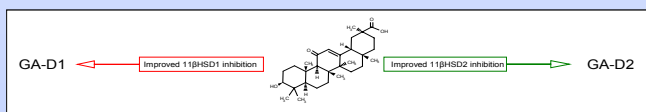
Inhibition of 11 β -HSD1 is considered a promising strategy to treat patients with metabolic syndrome by reducing glucocorticoid levels in adipose tissue, liver and skeletal muscle. Inhibition of 11 β -HSD2 was recently suggested for the treatment of inflammation and certain forms of cancer. *In vivo*, the ratio of cortisol to cortisone reflects the combined activities of 11 β -HSD1 and 11 β -HSD2. Thus, suitable cell assays to assess the selectivity of inhibitors and to investigate effects on glucocorticoid-mediated gene regulation are required.

Glycyrrhetic acid is a pentacyclic triterpenoid contained in different herbs such as liquorice. Glycyrrhetic acid acts as an inhibitor of both 11 β -HSD enzymes and represents a starting compound for the development of selective inhibitors.

PROCEDURE

STRATEGY:

Synthesis of Glycyrrhetic acid (GA) derivatives that are selective for either 11 β -HSD1 or 11 β -HSD2. (GA, GA-D1, GA-D2) Fig.2.



CONSTRUCTION OF CELL MODELS:

HEK-293 cells are devoid of endogenous 11 β -HSD and 17 β -HSD expression, therefore representing a suitable system to study effects on transfected enzymes at an identical background.

HEK-293 clones stably transfected with either 11 β -HSD1 and Hexose-6-Phosphat-Dehydrogenase (H6PDH) (HHH7) or 11 β -HSD2 (AT8) were constructed.

ANALYSIS OF THE POTENTIAL OF GA, GA-D1 and GA-D2 TO INHIBIT 11 β -HSD1 and 11 β -HSD2:

FUNCTIONAL ANALYSIS:

H4H1, liver cell line stably expressing 11 β -HSD1 was constructed for the analysis of the impact of inhibition on gene expression.

SW-620, human colon carcinoma cell line, stably expressing an NF- κ B reporter gene was constructed for the analysis of the anti-inflammatory effects of 11 β -HSD2 inhibition.

RESULTS

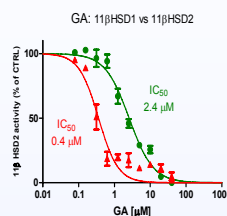


Fig. 3a

Glycyrrhetic acid (GA) inhibits both 11 β -HSD enzymes.

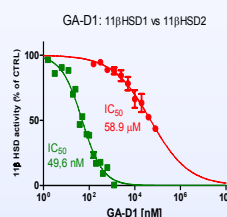


Fig. 3b

GA-D1 shows a >1000 fold higher selectivity for 11 β -HSD1 inhibition than for 11 β -HSD2.

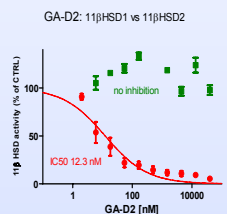
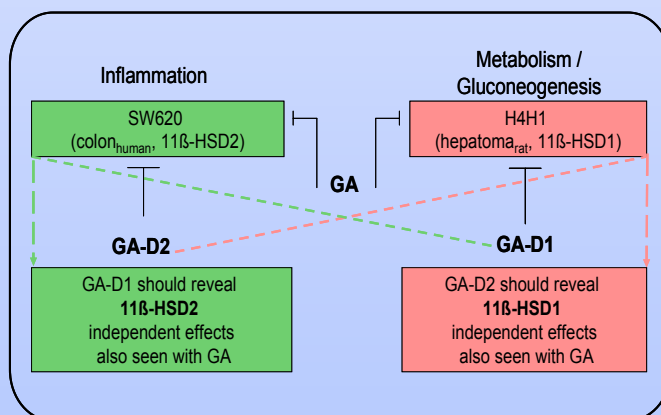


Fig. 3c

GA-D2 represents a selective 11 β -HSD2 inhibitor and shows no effect on 11 β -HSD1.

OUTLOOK

The three compounds (GA, GA-D1 and GA-D2) now allow to test the hypothesis that 11 β -HSD2 inhibition has beneficial effects in the treatment of inflammation and/or cancer. The compounds allow to distinguish between 11 β -HSD dependent and independent effects Fig.4.



SW-620 cells model for NF- κ B action and effects of 11 β -HSD2 inhibitor action on inflammation processes.

→ GA and GA-D2 should block, GA-D1 should have no effect.

H4H1 cells, model for effects of 11 β -HSD1 inhibitors on metabolic parameters, gluconeogenesis, gene expression.

→ GA, GA-D1 should block, GA-D2 should have no effect.

→ Identification of Off-target effects.